makes measurement of the width of the normally visible triple structure impossible, being closely apposed to the surface. However, when the membranes were distinct, their widths either conformed to those we report or were slightly greater.

The characteristic five-layered structure of mitochondrial cristae has been demonstrated by use of material fixed with potassium permanganate (*3*), as well as by freeze-substitution with a 2 percent solution of osmium tetroxide in acetone (*4*). The mitochondrial cristae, of tissues prepared by these two methods, show electron-microscopically three dark lines of similar density, of which the middle line is approximately 40 percent wider than the two outer ones. The mitochondrial cristae as seen in tissues processed with tetroxide (Fig. 4) show similar structure, but the middle electron-dense line is much wider relative to the outer lines and stains more deeply.

The membranes of the endoplasmic reticulum show occasional pores which may be the sites through which proteins and other macromolecules are transported. The electron-dense regions of the membranes may show, at high resolution, a trilaminar structure (Fig. 6) characterized by two electron-dense bands enclosing a slightly wider, less dense region.

Attempting to find a chemical basis for these differences between osmium and ruthenium tetroxides, we studied the reactivity of ruthenium tetroxide with tissue components. Using an ether extract of rat liver, separated chromatographically on silica gel, we found that ruthenium tetroxide reacts strongly with some of the more polar lipids that show no reaction with osmium tetroxide. Ruthenium tetroxide also reacts strongly with protein, glycogen, and the common monosaccharides, only slightly with mucopolysaccharides, and not strongly with the basement membranes in the tissues examined.

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Ecdysones and Analogs: Effects on Development and Reproduction of Insects

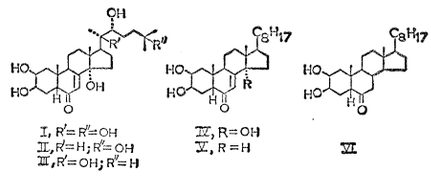
Abstract. *Ingestion of certain synthetic ecdysone analogs inhibited larval growth and development in several species of insects, whereas 20-hydroxyecdysone was inactive or considerably less active. Natural 20-hydroxyecdysone and ponasterone A, and a synthetic ecdysone analog inhibited ovarian maturation and egg production in the adult housefly. These effects appeared to be related to hormonal activity.*

The occurrence of steroids, with structures and biological activities similar to or identical with those of the molting hormones of insects, in various plants (*1*) poses a question of the roles of these substances in plants. It is not known whether these phytoecdysones are end products of sterol matabolism, analogous to the bile alcohols or acids of vertebrates, as suggested by the A/B ring *cis* configuration, or have a physiological or biochemical function in plant growth and development, as in insects. Of equal interest is their possible effect on insect host–plant relations. Desert locusts *Schistocerca gregaria* (Forskal) fed on moistened autumnal bracken-fern pinnae, plus wheat seedlings, took longer to develop and were smaller than locusts fed on wheat seedlings alone (*2*); it was suggested (*2*) that the effects reflected only dietary deficiency, and that it is unlikely that the plant ecdysones are a defense against insects.

We do not consider that this report (*2*) has answered the primary question of whether the ecdysones can act as plant protectants, or as regulators of insect host–plant interactions, because (i) although bracken contains ecdysones, including α-ecdysone and 20-hydroxyecdysone (*3*), at titers greater than the best insect sources, this fern is still a poorer source than are other plants (*1*); and (ii) testing for the effect of a minor component, by feeding the entire plant, is complicated by many factors—acceptance and ingestion of the plant by the insect, the overall nutritional value of the plant, and the presence of more than one toxic component.

We believe that this question can best be answered by determination of whether ecdysones and related steroids interfere with the normal development of insects when added to diets that otherwise support optimum growth and reproduction. We have therefore examined the effects of ecdysones and synthetic analogs by feeding them in nutritionally adequate diets.

Of the natural ecdysones, 20-hydroxyecdysone (I) was the most extensively studied, but α-ecdysone (II) and ponasterone A (III) also were tested in certain insects.



The synthetic steroids included three ecdysone analogs [Δ^7-5β-cholestene-2β,3β,14α-triol-6-one (IV), Δ^7-5β-cholestene-2β,3β-diol-6-one (V), and 5β-cholestane-2β,3β,diol-6-one (VI)] and their corresponding 5α-isomers (*4*). The test steroids were incorporated into the diets (*5*) by coating them on the dry components with volatile organic solvents. Five species of laboratory-reared insects were used: the tobacco hornworm *Manduca sexta* (Johannson), the flour beetle *Tribolium confusum* Jaquelin (duVal), the housefly *Musca domestica* (L.), the German cockroach *Blattella germanica* (L.), and the firebrat *Thermobia domestica* (Packard). Newly hatched larvae, or nymphs, and newly emerged adults were employed in the growth and reproduction tests, respectively.

Larvae of the housefly were very susceptible to the action of the hormone analogs: when added to an aseptic semidefined diet, compound IV severely inhibited development at concentrations as low as 37.5 parts per million (ppm) (Table 1). Removal of the 14α-hydroxyl group resulted in a compound (V) one-fourth as active in the housefly, but saturation of the Δ^7 double bond in V, to form VI, did not further reduce the activity. Ponasterone A was approximately one-fourth as active as compound IV, whereas 20-hydroxyecdysone was inactive at the highest concentration tested.

The various species showed considerable specificity in susceptibility to compound IV (Table 2). With the German cockroach and the flour beetle, removal of the 14α hydroxyl from IV, to form the diol (V), resulted in a compound about one-tenth as active. These two insects differed from the housefly in that further modification of compound V by saturation of the Δ^7 double bond, to form compound VI, further decreased activity so as to make the latter compound essentially inactive for both insects in our assay systems. The 20-

hydroxyecdysone was less than one-tenth as active as compound IV in the confused flour beetle; it was inactive in the tobacco hornworm at a concentration at which compound IV caused severe inhibition. Both α-ecdysone and 20-hydroxyecdysone were inactive in the German cockroach at 10 to 20 times the concentration of compound IV required to completely inhibit nymphal development. Thus the insect ecdysones only very slightly inhibited growth of immature insects.

Compound IV, 20-hydroxyecdysone, and ponasterone A all inhibited ovarian maturation and egg production when fed in an artificial diet to newly emerged houseflies. Their efficacies as female chemosterilants were compared by feeding them at different concentrations in artificial diets to groups each of 25 newly emerged female houseflies; after 4 to 5 days the terminal oocytes of the ovarioles were measured. Concentrations of 0.1 percent of compound IV severely inhibited ovarian growth (terminal oocyte was shorter than 0.3 mm) in about 80 percent of the flies; concentrations as low as 0.01 percent interfered with normal development of the oocytes. Approximately 0.5 percent of 20-hydroxyecdysone or 0.25 percent of ponasterone A equaled in inhibition 0.05 percent of compound IV. Inhibition by compound IV of ovarian development and egg production and hatching was only slightly reversed when the insects were subsequently fed an untreated diet.

Groups of newly emerged adult flour beetles were fed for 10 days on a diet containing 1.0 percent of compound IV; weekly for 8 weeks they were transferred to the untreated diet for 1 week, and the numbers of progeny were determined. Compound IV at 1.0 percent reduced the numbers of progeny by 97 percent, with no observable reversal of inhibition over the 8-week period. When males and females were fed separately on compound IV, only the female reproduction system was affected in both the housefly and the flour beetle.

For the following reasons we interpret the inhibition of reproduction and larval development as being related to the hormonal activity of these steriods:

1) Compound IV and the natural ecdysones, when fed to female houseflies, inhibited ovarian development and yolk deposition, suggesting an effect that is antagonistic to gonadotropic activity.

2) The hormonally inactive [molting-hormone assay (*6*)] 5α analogs of the synthetic steroids show little or no inhibition at concentrations of the 5β analog that show maximum inhibition —in some instances at even 10 to 20 times such concentrations.

Table 1. Effects of certain ecdysones and synthetic analogs on pupation and emergence of houseflies. Each percentage is based on four or more replicates, each of which consisted of 225 to 250 insects per 50 grams (wet weight) of medium.

Compound	Amount in diet (μg/g)	Percentages	
		Pupation	Adult emergence
Control	None	87	72
Compound IV	15	91	62
	37.5	67	12
	75	40	9
	150	29	4
Compound V	150	86	21
Compound VI	150	78	23
Ponasterone A	150	45	16
20-Hydroxy-ecdysone	150	85	69

Table 2. Ranges of dietary concentrations of compound IV required to kill or inhibit development in 75 percent of the test insects; at 10,000 μg/g it was ineffective in the firebrat.

Species	Range (μg/g)
Housefly	25 to 50
German cockroach	150 to 300
Confused flour beetle	500 to 750
Tobacco hornworm	750 to 1000

3) The effects of the active analogs on immature insects often proved to be related to molting and morphogenesis: repetitive molting in the tobacco hornworm, sclerotization and darkening of the housefly larva before formation of the puparium, interference with transformation from pupa to adult in the housefly and the flour beetle, and inability of the German cockroach to shed its exuvia during ecdysis.

This is the first report of inhibitive effects of ecdysones and synthetic analogs on growth of and reproduction by insects when these steroids are fed in the diet; the only earlier reports of comparable activity are that injections of certain ecdysones or synthetic analogs interfere with the normal molting of *Pyrrhocoris apterus* (L.) (*7*) and of dauer pupae of the silkworm *Bombyx mori* L. (*8*). Injections of several structurally diverse steroids, including certain 6-ketosteroid analogs, reportedly have a sterilizing effect on the female housefly (*9*).

Thus we have shown that ingestion of either synthetic ecdysone analogs or certain natural ecdysones severely disrupts growth or reproduction of several species of insects. The fact that topical application of several of our steroids proved ineffective indicates that there is uptake of these compounds from the intestinal tract. Some of our synthetic steroids showed activity at concentrations well within the concentrations of phytoecdysones in certain plants. The analogs, especially compounds IV and V, may well be similar to or even identical with intermediates in the biosynthesis of ecdysones; thus steroids resembling in structure the insect ecdysones, or intermediates in their biosynthesis in plants, may be more likely to serve as plant protectants against immature insects than the insect ecdysones per se. The wide range in susceptibility of species to the inhibition of larval development, by both the synthetic analogs and ecdysones, warns against generalizations or conclusions based on limited experimentation with a single species.

The compounds showing the highest molting-hormone activity in the housefly assay (*6*), the insect ecdysones, showed very low activity in the inhibition tests. In contrast, the growth-inhibitive synthetic analogs showed low activity in the molting-hormone assay. Thus the most active inhibitors may well be overlooked when only the classical molting-hormone assay is used in a search for natural ecdysone-like plant protectants; we now use inhibition tests in conjunction with such assays.

Aside from the possible role of the ecdysones in insect host–plant interactions, our results suggest practical implications and potential applications for these steroids. Certain synthetic analogs, with only minimum structural features of the insect ecdysones, were shown to inhibit severely growth and development in the housefly and other insects. The fact that feeding of either a synthetic analog or one of certain natural ecdysones inhibits ovarian development suggests them as models for the development of safe and specific antifertility agents for insects.

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13 August 1968

Teaching Effectiveness and Research Productivity

Bresler, in his article "Teaching effectiveness and government awards" (*1*), has presented data that seem to explode the myth, current in some quarters, that research productivity (as measured by publications and receipt of support from both external and internal sources) somehow detracts from teaching effectiveness in the classroom. The trends in general indicate that faculty members, whether in the sciences or the humanities, who are active and productive in research are perceived as more effective in their classroom performance than their colleagues who are not so active in research.

Notwithstanding the reasonableness of Bresler's final conclusion, there are several deficiencies in the data that seem to dilute the force of his argument. Some of these are deficiencies in the methods of collecting data—or at least in these methods as presented in the article—and others are deficiencies in the analysis of the data. (i) It is well known that many students take two or more courses within their own field in any given semester; hence, the returns used as the basis of computing means and standard deviations for faculty receiving research support as compared with those receiving no support, and so on, are not independent of each other. The degree of overlap between, or among, the samples should have been reported and discussed before any conclusions were drawn. (ii) No attempt was made to analyze the differences between means for various possible contrasts through use of a suitable statistical test. Although several of the differences are substantial, it is difficult for the reader to judge how dependable they are. (iii) On the basis of the information presented in Bresler's Tables 1 and 2, it is not possible to run any appropriate statistical tests, since it is not known whether the standard deviations Bresler reports represent variation within classes, variation across teachers' means, or some other estimate. Even if one assumes that Bresler's statistics are based on all ratings for all faculty in a category, it is still difficult to compute exact significance tests for contrasts across faculty groupings, since ratings in these categories concern unequal, and often highly disproportionate, numbers of faculty.

Thus, despite the fact that trends in the descriptive data tend to favor the conclusion that the college faculty active in research are judged by their students as superior in classroom performance to those of their colleagues who are less active in research, one can hardly consider this any more than a reasonable hypothesis worthy of proper experimental scrutiny and very likely to win confirmation.

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Reference

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My comments on Quereshi's three points, in order, are as follows: (i) His hypothesis of a departure from statistical independence is based on the assumption that there was systematic inter-rater bias. We cannot now determine whether or not such a bias was actually operative in the Tufts study. The summary student evaluations per course were available to me in a form which did not permit determination of the intercorrelation between the ratings given different courses by the same student. However (ii), the lack of independence—if there really was such a lack—would not affect the expected value of the mean scores but would affect the variance of the distribution of estimates. Since some students undoubtedly rated both supported and nonsupported faculty, the variance of the differences in means would be much less affected by correlated observations than by the variances of the means themselves. The data ought to be viewed as a descriptive case study of the performance of Tufts University. A significance test would not add much. (iii) It is not clear whether Quereshi uses the word *classes* to represent courses or statistical groups. If the latter, the answer, of course, is variation within classes. In Table 1 of my article, 640 represents the number of student returns for 15 courses taught by 13 faculty members.

Quereshi's sympathetic concluding statement about "experimental scrutiny" prompts these further comments. Reflection will show that a study such as the one undertaken at Tufts cannot be made in the overwhelming majority of American universities and colleges because at most such institutions there are simply not enough faculty members holding government awards to provide an adequate test. Conversely, in the top-ranking 20 or 30 universities, such a large number of the faculty members hold such awards that it would be equally difficult to make adequate tests in these institutions.

One should have at least 15 faculty members in each cell under evaluation in a two-by-three arrangement representing support versus no support, and representing sciences, social sciences, and arts and humanities.

This by no means exhausts the conditions for a "proper experimental scrutiny." Yet, due to Tufts University's position in the select company of the 100 institutions receiving the most government funds, even these basic requirements were not totally met.

There are very few institutions in the United States where a study under "proper experimental scrutiny" could be made at this time. Hence, I, like Quereshi, would welcome further data, even though fragmentary, on a problem which has evoked much heat.

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